Evaluation of Erythrocyte and Thrombocyte Parameters in Pediatric Patients with Diabetes Mellitus

Diabetes Mellitusu olan Çocuk Hastalarda Eritrosit ve Trombosit Parametrelerinin Değerlendirilmesi

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Özet

Amaç: Bu çalışmayı yapmaktaki amacımız, diabet, diabetik ketoasidoz ve kontrol hasta grubunda eritrosit ve trombosit parametrelerini değerlendirmektir. Gereç ve Yöntem: 2015 Ocak ve 2016 ocak tarihleri arasında çocuk acil kliniğinde diabetik ketoasidoz(DKA) tanısı alan hastalar Grup 1, rutin kontrol amacıyla pediatrik endokrinoloji polikliniğine başvuran ve ketoasidoz tablosunda olmayan tip 1 diabetli hastalar(DM) Grup 2, ve kontrol hastaları Grup 3 olarak adlandırıldı. Bulgular: Çalışmaya 36'sı "DKA", 37'si "DM" ve 35'i "Kontrol" olmak üzere toplam 108 hasta alındı. Kontrol grubunun MPV ortalaması, DKA ve DM gruplarından anlamlı şekilde düşük idi ($p<0.001$). DKA grubunun MCHC ortalaması, DM grubundan anlamlı şekilde daha yüksekti ($p:0.017$). PDW ortalaması, DM ve kontrol gruplarından yüksek bulundu ($p<0.01$). Gruplar arasında RDW, MCV ve RDW/MCV düzeyleri açısından istatistiksel olarak anlamlı bir farklılık bulunmadı ($p>0.05$). Tartışma: İnflamatuvar bir marker olan MPV'nin DKA ve diabet grubunda normal populasyona göre daha yüksek olduğu saptandı. MPV, RDW, PDW ve MCHC yüksekliğinin DKA görülme riskini arttırdığı bulundu.

Anahtar Kelimeler

Diabetes; Ortalama Trombosit Hacmi; Trombosit Dağılım Hacmi; Eritrosit Dağılım Hacmi

Abstract

Aim: To analyze erythrocyte and thrombocyte parameters in patients with diabetes, diabetic ketoacidosis, and controls. Material and Method: Patients who were diagnosed as having diabetic ketoacidosis (DKA) in the pediatric emergency clinic between January 2015 and January 2016 were included in group 1; patients with type 1 diabetes mellitus (DM) who consulted the pediatric endocrinology outpatient clinic for routine controls were included in group 2, and control patients were in group 3. Results: A total of 108 patients comprising 36 patients with DKA, 37 patients with DM, and 35 controls were involved in the study. The mean platelet volume (MPV) of the control group was significantly lower than the DKA ($p= 0.001$) and DM ($p= 0.001$) groups ($p<0.01$). The mean corpuscular hemoglobin concentration (MCHC) was significantly higher in the DKA group than in the DM group ($p= 0.017$), and the mean value of platelet distribution width (PDW) in the DKA group was found higher than that in the DM and control groups ($p<0.01$). There was no statistically significant difference between the groups in terms of red cell distribution width (RDW), mean corpuscular volume (MCV), and red blood cell distribution width/mean corpuscular volume (RDW/MCV) levels ($p>0.05$). Discussion: An inflammatory marker, MPV, was found higher in the DKA and diabetic groups than in the normal population. High levels of MPV, RDW, PDW, and MCHC increased the risk of DKA incidence.

Keywords

Diabetes; Mean Platelet Volume; Platelet Distribution Width; Red Cell Distribution Width

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Introduction

One of the frequent chronic diseases in childhood, insulin-dependent type 1 diabetes mellitus is a vital public health problem because of its complications that can develop in the long term. Its incidence under the age of fourteen ranges from 0.1/100,000 to 36.8/100,000 annually, and differs between countries [1]. The global prevalence of diabetes is increasing rapidly, and it is estimated that there will be 592 million patients with diabetes worldwide in 2035 [2].

Mean thrombocyte volume is a significant marker that informs about function and activation of thrombocytes. Large-volume platelets are metabolically more active and more prone to adhesion and aggregation. Oscillations of thromboxane A2, serotonin, platelet factor 4, thrombocyte factor 4, and β-thromboglobulin of large thrombocytes are augmented. Many studies have demonstrated increased levels of mean platelet volume (MPV) in cases of types 1 and 2 diabetes mellitus (DM), impaired fasting glucose, insulin resistance, hypertension, hyperlipidemia, and metabolic syndrome [3, 4].

In this study, we aimed to evaluate erythrocyte and thrombocyte parameters in diabetic, diabetic ketoacidosis, and control groups.

Material and Method

This study was performed retrospectively after Ethics Committee approval was obtained.

Patients who were diagnosed as having diabetic ketoacidosis (DKA) in the pediatric emergency clinic between January 2015 and January 2016 were included in group 1. Patients with type 1 diabetes mellitus who consulted the pediatric endocrinology outpatient clinic for routine controls were included in group 2, and patients who were hospitalized in the pediatric surgery center for minor surgical interventions were assigned to group 3. The World Health Organization (WHO) criteria were taken as a basis for the diagnosis of type 1 diabetes mellitus. During admission, detection of hyperglycemia, ketonuria, and cases of venous pH <7.3 and HCO3 <15 mEq/L were considered as indicators of DKA [5]. The patients' sex, age, reasons for referral to hospital, and clinical features were analyzed. Patients were excluded from the study if they had insufficient data, idiopathic thrombocytopenic purpura, thrombocyte function disorders, thrombosis, malignancy and hypersplenism, received platelet transfusion, bleeding throughout the study, immunodeficiency, had taken aspirin, heparin, received chemotherapy, received immunosuppressive therapy apart from steroids, had infections, or were over the age of 16 years. The patients' age, sex, the time in which they were monitored with the diagnosis of DM, complete blood count values at the time of referral, blood glucose, and HbA1c levels were recorded.

The complete blood count results were studied within one hour using K3 EDTA and anticoagulated blood samples using an Abbott Cell-Dyn 3700 (Abbott Diagnostics, Santa Clara, CA, USA). The platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), mean corpuscular hemoglobin concentration, red cell distribution width (RDW), mean corpuscular volume (MCV), and RDW/MCV were recorded. Hemoglobin A1c (HbA1c) measurements were analyzed using ion-exchange chromatography (TOSOH, G8).

Statistical Analysis

IBM SPSS Statistics 22.0 (IBM SPSS, Turkey) was used for the statistical analyses. The suitability of the parameters of the study data for normal distribution was evaluated using the Shapiro-Wilk test. In addition to descriptive statistical methods (mean, standard deviation, frequency) during the evaluation of the study data, the one-way ANOVA test was used to compare the quantitative data and normally-distributed parameters between the groups, and Tukey's HDS test was used to detect the group that caused the difference. For the parameters with no normal distribution, the groups were compared using the Kruskal-Wallis test and the group that caused the difference was detected using the

Mann-Whitney U test. The parameters with normal distribution were compared within two groups using Student's t-test, whereas the parameters with non-normal distribution were compared within the two groups using the Mann-Whitney U test. In comparison of the qualitative data, a Chi-square test was conducted. Logistic analysis was performed for the multivariate analyses. Pearson's correlation analysis was used to assess the relationships between the parameters that showed normal distribution and Spearman's rho correlation analysis was performed to analyze the relationships between the parameters that did not show normal distribution. Significance was evaluated as $p<0.05$.

Results

A total of 108 patients aged between 13 and 190 months consisting of 57 (52.8%) female and 51 (47.2%) male patients were enrolled in the study between January 2015 and January 2016. The patients were analyzed in three groups as the DKA (n=36), DM (n=37), and control (n=35) groups. There was no statistical difference between the groups in terms of mean age and sex. The mean age and sex distribution of the patients in the groups are shown in Table 1.

Table 1. Age and Sex Distribution of the Groups

	DKA	DM	Control	p
$Age_{Mn\pm SD}$	124.11±48.60	118.32±43.21	101.89±49.86	[1]0.126
$Sex_{n,\%}$				
Female	21 (58.3%)	16 (43.2%)	20 (57.1%)	[2]0.356
Male	15 (41.7%)	21 (56.8%)	15 (42.9%)	

[1]One-way ANOVA Test [2]Chi-square test

The time of diagnosis was shorter (p= 0.001), the blood glucose and HbA1c levels were higher (p= 0.001), and the pH and HCO3 levels were significantly lower in the DKA group than in the DM group (p<0.01) (Table 2).

There were statistically significant differences between the groups in terms of the MPV, MCHC, and PDW levels (p= 0.001, p= 0.023, p= 0.001, respectively). The MPV of the control group was significantly lower than that of the DKA (p= 0.001) and DM (p= 0.001) groups (p<0.01). There was no significant difference found between the DKA and DM groups regarding MPV levels (p>0.05).

The MCHC was significantly higher in the DKA group than the DM group (p= 0.017). There was no significant difference between the other groups in terms of MCHC levels (p>0.05). The

Table 2. Assessment of the DKA and DM groups regarding time of diagnosis, serum glucose, HbA1c, pH, and HCO3

	DKA	DM	p
	Mn±SD	Mn±SD	
Time of diagnosis (year) $_{(median)}$	9.72±17.75	14.05±22.77	[1]0.001**
Blood glucose(mg/dL)	545.28±131.33	173.7±53.74	[2]0.001**
HbA1c(%)	11.65±2.61	8.64±2.07	[2]0.001**
pH	7.22±0.13	7.38±0.04	[2]0.001**
HCO3(mEq/L)	13.44±6.37	23.29±2.22	[2]0.001**

[1]Mann-Whitney U test [2]Student's t-test **p<0.01

mean PDW in the DKA group was significantly higher compared with the DM (p= 0.001) and control (p= 0.001) groups (p<0.01). There was no difference between the DM and control groups in terms of mean PDW values (p>0.05).

There was no statistically significant difference found between the groups in terms of the RDW, MCV, and RDW/MCV levels (p>0.05) (Table 3).

Table 3. Assessment of the Groups Regarding PLT, MPV, MCHC, PDW, RDW, MCV, and RDW/MCV

	DKA	DM	Control	p
	Mn±SD	Mn±SD	Mn±SD	
PLT(/mm^3)	308.86±72.11	277.76±60.86	333.26±96.39	[1]0.012*
MPV(fL)	7.82±1.09	7.82±1.05	6.95±0.73	[1]0.001**
MCHC(gr/dL)	34.16±1.45	33.45±0.78	33.78±0.93	[1]0.023*
PDW$_{(median)}$	19.52±7.23 (17.8)	17.29±0.95 (17.3)	17.01±0.6 (17)	[2]0.001**
RDW(%)	16.23±1.56	15.46±1.25	15.61±1.61	[1]0.069
MCV(fL)	81.99±4.53	81.78±5.71	80.5±5.11	[1]0.421
RDW/MCV	0.19±0.02	0.18±0.02	0.19±0.03	[1]0.304

1One-way ANOVA Test 2Kruskal-Wallis test *p<0.05 **p<0.01

There was no statistically significant relationship in the DKA group between the time of diagnosis and PLT, MPV, MCHC, PDW, RDW, MCV, and RDW/MCV (p>0.05). In the DM group, there was a positively-oriented 32.2% statistically significant relationship found between time of diagnosis and MCV (p= 0.049; p<0.05). There was a negatively-oriented 39% statistically significant relationship between time of diagnosis and RDW/MCV (p= 0.017; p<0.05). There was no statistically significant relationship found between time of diagnosis and PLT, MPV, MCHC, PDW, RDW (p>0.05).

When we analyzed the effects of PLT, MPV, MCHC, PDW, RDW, MCV and RDW/MCV parameters on formation of DKA using backward stepwise logistic regression analysis, we discovered that the model was significant (p<0.001), the Nagelkerke R square value was 0.586, and the explanatory power of the model (78.9%) was at a favorable level. The effects of the MPV, MCHC, PDW, and RDW parameters in the model were statistically significant (p<0.05; p<0.01). A high level of MPV multiplied the risk of DKA incidence by 4.129.

It was determined that the risk of DKA incidence increased by 4.129 times with a high level of MPV, by 2.724 times with a high level of MCHC, by 3.238 with a high level of PDW, and by 1.905 with a high level of RDW.

Table 4. Evaluation of the Effects of PLT, MPV, MCHC, PDW, RDW, MCV, and RDW/MCV Parameters on DKA Incidence in Logistic Regression Analysis

	OR	95% Confidence Interval		p
		Lower Limit	Upper Limit	
MPV	4.129	1.506	11.320	0.006**
MCHC	2.724	1.176	6.311	0.019*
PDW	3.238	1.141	9.190	0.027*
RDW	1.905	1.192	3.046	0.007**

PLT, MPV, MCHC, PDW, RDW, MCV, and RDW/MCV parameters were included in the model. *p<0.05 **p<0.01

Discussion

It is an established fact that diabetes is a prothrombotic condition. Acquired thrombophilia often develops due to a dysfunction in homeostasis. Thrombocytes are fundamental components of the atherothrombotic process owing to their prothrombotic and proinflammatory functions. Patients with diabetes are exposed to increased platelet reactivity; its cause is multifactorial. Both metabolic reasons such as hyperglycemia, hypertriglyceridemia, and neurohormonal activation and systemic reasons such as oxidative stress, inflammation, and insulin resistance can be considered amongst these factors [6,7].

Hyperglycemia induces nonenzymatic glycosylation of proteins on the surface of the platelet, and therefore, reduction in membrane fluidity and increase in platelet reactivity.

Hyperglycemia also has direct osmotic effects on platelets. Additionally, this increase in the MPV value might also be caused by the rise in consumption of platelets and production of young platelets [8,9]. Similarly, hyperglycemia has effects on erythrocytes. It decreases deformability and changes the mechanical characteristics of erythrocytes while increasing osmotic fragility and adhesion, causing changes in the structure and hemodynamic characteristics of erythrocytes, and effectively decreasing lifespan. Peterson et al. [10] discovered that there was an extension in lifespan after near-normal glycemic control and this extension was significantly higher for poorly-controlled patients.

It is known that RDW is an independent indicator for mortality in both general population and high-risk groups (e.g. obesity, chronic kidney disease, heart failure). High RDW levels indicate cell size heterogeneity, namely anisocytosis. RDW is a parameter that is used along with MCV in investigating the etiology of anemia [11, 12].

Inflammation erythropoiesis might affect erythrocytes' half-life in circulation and erythrocyte deformability, and cause anisocytosis. The mechanism explaining the relationship between diabetes and RDW is not clear [13]. We discovered in our study that a high level of RDW increases incidence of DKA by 1.905.

Liu et al. [14] investigated erythrocyte parameter changes in patients with diabetes. They reported that the patients with DKA had higher red cell distribution width (RDW) and RDW/MCV rates compared with patients who were non-DKA and with the control group. They discovered RDW was returned to normal after plasma glucose levels and metabolic acidosis were controlled. In a similar study conducted in 2015, it was reported that patients with diabetes had distinctively higher values of RDW compared with healthy individuals, and anisocytosis developed due to impaired erythropoiesis, agglutination, or eryth-

rocyte degranulation with fragmentation [15]. Conversely, we found no difference in the RDW, MCV, and RDW/MCV values between groups in our study ($p<0.05$).

Hekimsoy et al. [16] discovered that the patients with type 2 diabetes had higher MPV but lower mean number of platelets compared with the control group. In a similar study, Papanas et al. [17] reported that the diabetic patient group with or without complications had higher MPV values than the control group. In a retrospective study in 2015 that included 4072 patients, there was a positive relationship reported between HbA1c level and MPV [18].

In another study conducted in 2013, patients with diabetic ketoacidosis had significantly higher plasma glucose levels, HbA1c levels, MPV, and PDW values than the patients with diabetes without ketoacidosis and with the healthy controls. With the results from the logistic regression analysis, it was reported that a high PDW could pose a risk factor for the presence of DKA [19]. Similarly, the MPV values in the DKA and DM groups were significantly higher compared with the control group in our study ($p<0.01$). There was no significant difference between the DKA and DM groups in terms of MPV ($p>0.05$). Concordantly with the literature, there was a statistically significant difference between the groups in terms of PDW levels; the mean PDW of the DKA group was significantly higher than the DM group ($p= 0.001$) and controls ($p= 0.001$) ($p<0.01$). We determined that the risk of DKA incidence increased by 4.129 times with a high level of MPV, by 2.724 times with a high level of MCHC, by 3.238 with a high level of PDW, and by 1.905 with a high level of RDW. Kodiatte et al. [20] reported that MPV levels of patients with diabetes were significantly higher compared with healthy individuals. They found no difference in MPV levels of patients with diabetes with complications such as hypertension, coronary artery disease, peripheral arterial disease, hyperlipidemia, diabetic neuropathy, nephropathy, and retinopathy and uncomplicated patients; however, they found a positive relationship between MPV and HbA1c.

Similarly, Demirtunç et al. [21] found higher MPV levels in patients with DM. They reported that patients with HbA1c >7 had distinctively higher levels of MPV than patients with HbA1c ≤7. However, three months after good glycemic control, they detected a significant reduction in MPV compared with base line, and reported that MPV activity could be used in glycemic control monitoring, and therefore could play a role in the prevention of complications. In another study conducted in 2016, it was remarked that every 1 femtoliter increase in MPV was positively correlated with twice-insufficient glycemic control. In the study, which was performed with adult patients, the sensitivity was 82% and the specificity was 54.5% for the MPV 9.55 fL cut-off value [22]. In another similar study, the decrease in HbA1c and the decrease in thrombus formation were reported to have a distinct positive correlation [23].

There have been more studies that focus on the relationship between type 2 diabetes and MPV. In a study that was conducted with pediatric patients with type 1 diabetes in 2015, it was demonstrated that the diabetic patient group had higher MPV, RDW, and platelet large cell ratio (P-LCR) than the control group [24].

Consequently, MPV, an inflammatory marker, was found in higher levels in the DKA and diabetic groups than in the normal population. High levels of MPV, RDW, PDW, and MCHC increased the risk of DKA incidence.

Competing interests

The authors declare that they have no competing interests.

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